

Exhibit I

N-acetyl Hexosamine Acid (Sialic Acid)

NeuNAc α 2,3Galβ1,4GlcNAc 2'Sialic acid NAe

(β1,4 GalNAcT)

F + II (3,5,7)

NeuNAc α 2,3Galβ1,4GlcNAc

↑ β1,4 /

GalNAc

NeuNAc α 2,3Galβ1,4GlcNAc

↑ 3α

Fucose

To Prepare TEA (HPLC)

2 M (TEA) Sulfate pH 5.5 (one liter)

550 ml H₂O

120 ml Glacial Acetic Acid (120/liter - 2M)

Titrate w/ Triethylamine to 5.5

150+ mls

Bring to 1 liter - filter thru Millipore filters
(designed for HPLC reagents)

FT7 assays

Item		F
0.5 1M Na-Cacodylate		3.5
0.2 2M NHCl ₂		1.4
0.1 500mM L-fucose		2.8
1.0 100mM ATP		7.0
.8 ¹⁴ C 6DP-fucose		5.6
H ₂ O 9/20		35.7

Stock of
Sialyl α 2,3GalNAc Fw = 675

50mM = 29.07 / 1 mg

Cocktail

	Cocktail	Extract	H ₂ O - Acc	H ₂ O + Acc (SLN 50mM)
FT3 DL	8.0	4.0	8	6 2
FT3 BIC	8.0	4.0	8	6 2
FT7 SN	8.0	10.0	2	0 2

In Rxn 3 hrs 37°C, Partition w/ NaH₂PO₄ Column count

Sulfated Acceptor

Spd of 20 μl rxn

6.14 mg/100 μl 18.7 μl = 2.3 mg

WED

14:05

Reaction counts

Appear to have
Worked

PARAMETER GROUP: 1
ID: 14-C 1 MIN

Dry down remaining
portion of fm
& Put on HPLC

POS	CTIME	CPM1
001	00060	734.57 F13 DL + Acc
002	00060	398.40 F13 DL -
003	00060	1617.20 F13 BK +
004	00060	518.50 F13 BK -
005	00060	1185.50 F17 +
006	00060	368.40 F17 -

POS	CTIME	CPM1
011	00060	468.50
012	00060	425.40
013	00060	417.96
014	00060	517.50
015	00060	482.50
016	00060	487.50

TOTAL COUNT RATE: 7621.9 CPM

]
TOTAL
COUNTS
5ul

From the HPLC Data

the counts in FT7 (SN)

Sample are only facose
this E prep is inactive

Cells were transferred on 1/27 w/ mFT7 (1,2ab,3) and hFT3 (JBC 125)

Cells were FACS Analyzed on 1/28 - 4 plates each

Stained w/ Lx ; SLK + H

	H	SLK	Lx
mFT7	-	+	-
hFT3	-	+	+

as would expect

Made cell 1% Triton extract portion off
Fucose Transfase with SCN as acceptor

Cocktail Extract H₂O - + H₂O

FT3 10.5 7.5 2 2

FT7 10.5 7.5 2 2

Previous FT3(B) 10.5 4.0 5.5 2 3.5

Cocktail 4 times

0.5 Mgl Calo 2.0

0.2 Mmgl 0.8

0.4 L-fucose 1.6

1.0 A+P 4.0

0.8 6D P fucose 3.4

10.4 H₂O 40.4

All runs worked fine

Use this extract to
check out Sulfated
acceptor
and run these
on the HPLC

PARAMETER GROUP: 1
ID: 14-C 1 MIN

POS	CTIME	CPM1
001	00060	3677.60 FT3B
002	00060	7989.80 FT3 (nm)
003	00060	1651.20 FT7
004	00060	746.80 - am
005	00060	1945.80 - am
006	00060	679.70 - am
007	00060	454.50
008	00060	457.50
009	00060	474.50
010	00060	454.50
011	00060	409.40
012	00060	442.40

Set of 20% AF
added to 250µl
Count 10

TOTAL COUNT RATE: 18383.8 CPM

HPLC runs

Mock

Fucose

6DP fucose

SLx (Standard)

FT3 (new) rxn product

in FT7 reaction product

- product migrants of
SLx

Run FT assays w/ FT3 and SLN and Sulfated
acceptor. The sulfated acceptor will not
be recovered over NaH₂PO₄ Columns, analysis
will be thru the HPLC separation

FT assay	cocktail	50mM		50mM		H ₂ O
		Cocktail	Cytoact	SLN	Sul	
FT3-S1	Na ₂ CO ₃ 3.5	13	5.0	2.0		
Sul	MgCl ₂ 1.4	13	5.0		2.0	
S2	Cfuc 2.8	13	5.0	2.0		
-	ATP 7.0	13	5.0			
	6DPfuc 5.6					
	H ₂ O 53.2					

HPLC Analysis

Mock

Fucose

6DP fucose

SLx

FT3/SLN FT7 SLN

Acceptors
(final) 5mM